

REMARKS

This is in response to the office action mailed February 25, 2003. A Petition for a one month extension of time with fee is enclosed herewith.

Claims 1 to 26 are pending in this application. Claims 1, 8, 12, 13, 14 and 15 have been amended. Claims 20 to 25 are withdrawn from consideration. Based on the amendments as well as the discussion herein, favorable reconsideration of all of the claims is requested.

Rejection under 35 U.S.C. 112

The claims have been rejected under 35 U.S.C. 112, second paragraph, for reasons set out on pages 2 and 3 of the office action. Applicants specific response to the rejections are as follows:

Re claim 1, line 8: The phrase "an effective amount" has now been clarified. The claim now states that the surfactancy is "no more than 5% v/v to allow antigens to attach to the beads" so that it is no longer vague and indefinite. As is well known, "v/v" is the abbreviation for "volume to volume" and in this case means that 5% of the total volume of liquid in which the beads are suspended is surfactant.

Re claim 1, line 6: The phrase in the claim "including washing the beads in the buffer" is neither unclear nor inconsistent. The Examiner is requested to note that the method of the invention is one for "making a no wash bead based assay", and the method includes a bead washing step. This does not mean that the no wash bead based assay made in accordance with the method of claim 1 involves a "wash" step, which it does not.

Re claim 1, line 11: The Examiner's point is noted and a corrective amendment introduced in this Response.

Re claim 8, line 2: The Examiner's point is noted and a corrective amendment introduced in this Response.

Re claim 12: Applicant requests clarification of this rejection. As far as Applicant is concerned, each recited substance is clearly specified.

Re claim 13: (1) The Examiner's point is noted and a corrective amendment introduced in this Response. (2) As regards the "antibody/antigen" issue, the Examiner is advised that it is well known that these terms can be used interchangeably, since an antigen may in certain contexts be an antibody and vice versa. In claim 13, for example, the bead contains (i.e. is coated with)

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antigens to specific bacterial/viral proteins. That system is then used to capture (detect) certain bacterial/viral antibodies. However, once you utilize a "detector" antibody (e.g. goat anti-human IgG FITC, etc.) for the purpose of identifying the presence of bound antibody to that specific antigen, the "detector" antibody itself is looking for a specific antigen which, in this case, is another antibody. Thus, these terms are often interchangeable depending on their context.

Re claim 14: Please see comments with respect to claim 1, line 8 above.

Re claim 26: Note that claim 26 is not a method claim, but an assay or "test kit" produced by the method fo claim 1. Therefore, active steps need not be recited. To emphasize this point, Applicant would be willing to describe the assay as "bead based assay composition" if this would clarify the matter for the Examiner.

Rejection under 35 U.S.C. 102

Claims 1, 5 to 11, 13 to 19 and 26 are rejected under 35 U.S.C. 102(b) as anticipated by Hansen. It is submitted that the discussion below relating to the patentably distinguishing features of the invention will clearly show this reference not to be

relevant.

Rejection under 35 U.S.C. 103

Claims 2, 3, and 4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hansen in view of Fulwyler. Claim 12 is rejected under the same provision as being unpatentable over Hansen in view of Lim. Once more, it is submitted that the discussion below relating to the patentably distinguishing features of the invention will clearly show this reference not to be relevant.

The claimed invention

Before discussing the references, it is appropriate to point out the claimed specific steps of the method of the invention, the order of the steps and the effect of each step in formulating the no wash bead assay. The Examiner is requested to bear in mind that the claimed invention is not a random arrangement of general components, but is a combination of specifically directed components in a particular order. This fact must not be lost sight of, since it produces an assay having properties and characteristics which are vastly different from that of the cited references.

*Claim 1* thus requires the following components in the specified order for the *method of making a no wash bead based*

assay:

- (1) A first reagent comprising a *buffer* is prepared.
- (2) A second reagent comprising a *protein* is prepared.
- (3) *Beads* of preselected size and having a coefficient of variation less than 5% are prepared. This preparation step includes washing the beads in the buffer to form a *bead-buffer matrix* and *reducing the surfactancy* of the beads to no more than 5% v/v to allow antigens to attach to the beads
- (4) An *antigen* for detecting the presence of a target species is added to the bead-buffer matrix such that the antigen attaches to the beads to form a *bead-antigen mixture*, the surfactancy of the beads facilitating attachment of the antigen thereto.
- (5) The first reagent buffer is added to the bead-antigen mixture and the *mixture* is thereafter incubated.
- (6) The second reagent is added to the bead-antigen mixture to *reduce or eliminate non-specific binding sites*.

Neither Hansen nor Fulwyler, individually or in combination, discloses such a specific set of steps with the components recited. In the office action, the Examiner parcels together disparate and, it is submitted, unrelated bits and pieces from Hansen (on page 4), but even the very points made by the Examiner utterly fail to add up to the invention as claimed. The Examiner is requested to not

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lose sight of the *claimed invention*, and to refrain from creating a patchwork of passages from Hansen, based on hindsight, which do not support the rejection.

It will be seen upon reviewing the summary of claim 1 above that there are a large number of elements and steps where both Hansen and Fulwyler totally fail to teach or even hint at the claimed invention.

#### Discussion of Hansen

The process described in this patent is unlike anything in the claimed invention. Hansen utilizes the process of *agglutination of beads* to create complexes of 2, 3, or more *clumps* and distinguishes them by their scatter properties. This is actually a good example illustrating the difficulty encountered in the early stages of bead based assays, namely, that investigators had real problems with bead clumping and had to live with it as an analytical tool. The presently claimed invention has virtually eliminated the clumping issue and uses pure scatter properties to distinguish the bead populations. Applicant is able to discretely separate individual, single bead sizes because of the unique properties of the coating and analysis procedures as set forth in the claims.

As quoted in Hansen, "the present invention relates to optical

analytical methods based on rates of particle agglutination" (see column 1, lines 10 to 12). Hansen uses (Ab)-antigen A present in solution, which will couple beads containing antibodies to antigen A forming a bead complex. Likewise, the same is true for the detection of antibodies in samples to antigens.

OK The Examiner mentions that this invention is a "no-wash" system and questions why there is washing in the initial portions of the application. As mentioned above, it should be realized that the part he is referring to is the *manufacturing procedure* and the assay does require washing to elute-off any excess antigens not bound to the surface of the beads. There are no washing steps in the actual analytical portion using the invention.

\* Hansen fails to sequentially, or otherwise, define the buffer, the protein, the bead-buffer matrix, adding the antigen to form the bead-antigen mixture, and then adding the buffer and reagent to incubate and reduce non-specific binding sites respectively. There are many procedures well known which use beads, washing, incubation etc. But Applicant claims a specific sequentially relevant method.

✓ It is also pointed out that the coefficient of variation of bead size, and the reducing of surfactancy, are two different events in the method. The Examiner seems to suggest in the office

action that these are the same by stating that Hansen "teaches selecting the size of beads around coefficient of variation ... to reduce surfactancy of the beads". The bead size variation and the surfactancy levels are separate and discrete elements in the claims and each should be accorded its own weight in defining the method of the invention.

#### Fulwyler

Applicant has developed a manner of coating the beads which is unique compared to the procedures in Fulwyler. Some of these difference are:

(1) Applicant does not use PBS at pH 7.0 or Tris Buffer pH 8.4. Even though buffer in appendix states making carbonate buffer pH 9.5, ours is bicarbonate and carbonate specialized mixtures.

(2) Applicant does not need PBS-BSA-Tween to wash the beads. This generally is used to prevent clumping. We have beads *formulated prior to coating that eliminates this problem* (see surfactancy component).. Not claimed!

(3) Applicant requires only a protein/bead incubation in the refrigerator for 12 - 18 hours. No pre-incubation as in Fulwyler at 37 degrees for 3 hours is required. Not claimed.

(4) No 37 degree incubation for 1 hour for blocking is required. Applicant's invention is a one wash step with a low percentage of BSA in carbonate buffer.

(5) Storage - Applicant does not need to store beads in solutions containing glycerol. Applicant does not need to pre-wash the beads prior to usage.

(6) Applicant's invention is a "no-wash" assay (i.e. no washes between incubation steps), which is not the case with Fulwyler.

Lim has nothing to do with flow cytometry. It describes a "flow through" system which looked at doublets and was spectrophotometrically based (wave light absorbance).

#### General Comments

This invention is far advanced from any patent or article cited and uses techniques that are not obvious to those skilled in the field. It has taken much effort and experimentation to develop the system, which is unlike any currently cited. Extensive research has gone into the types of beads, optimizing the percentage of surfactants in the storage medium, and optimal binding conditions for each type of antigen/antibody reaction. Furthermore, the ability to prevent the beads from clumping (i.e. forming complexes of 2 or more beads) is very significant and difficult to achieve, and different from any of the other procedures mentioned. Applicant's invention has accomplished a way to virtually eliminate this phenomenon, unlike Hansen's patent

whose entire premise is based on *agglutination*, the very antithesis of the method claimed in the present invention.

Further, Hansen utilizes light scatter properties to detect the aggregates of the beads. This only implements two of the possible analysis channels found in modern flow cytometers. He also uses a "Delta" difference in the refractive index between reactions occurring in the bead signals' to determine differences, or variations, of the singlets versus the doublets and triplets or multiplexes of beads. This "Delta" is the measure of positivity.

→ The claimed invention uses size discrimination for separating each bead and then fluorescent markers to detect the presence or absence of the specific binding of the antigen or antibody to the bound antigen or antibody, respectively. Hansen has limitations on the number of assays performed in one tube because of the interference agglutination may cause using multiple sized beads. The present invention uses discrete sizes which are easily recognizable and, therefore, have the ability to separately be distinguished one bead assay from the other. This greatly facilitates user operation and interpretation.

It is hoped that the above will assist the Examiner in understanding the significant patentable features of the invention, and favorable reconsideration of all the claims and allowance of

June 25, 2003


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the application is therefore respectfully solicited.

If the Examiner has any questions, she is invited to contact the undersigned at (818)710-2788.

Please acknowledge receipt hereof by stamping and returning the enclosed return postcard.

Respectfully submitted,



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Enclosed: Petition for extension  
Check  
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Certificate of Mailing (37 CFR 1.8):

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450, on June 25, 2003.



Colin P. Abrahams